SUPPORTING INFORMATION

Divergent aquifer biogeochemical systems converge on similar and unexpected Cr(VI) reduction products

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Hanford 100H custom microarray design

As described briefly in the main text, a custom NimbleGen microarray (6 x 630K format) was designed to analyze gene expression of Hanford 100H aquifer microbial communities under conditions relevant to chromate reductive immobilization with lactate as the electron donor. Microarray probes were based on the predicted open reading frames (ORFs) from genomes of three Hanford 100H bacterial isolates that catalyze chromate reduction (*Pelosinus* sp. strain HCF1[1], *Pseudomonas* sp. strain HCN1, and *Desulfosporosinus* sp. strain HCS1) and 12 metagenomes from various Hanford 100H experimental systems, including denitrifying and fermentative columns (described here) and groundwater samples from field tests involving lactate injection. 222.7 million reads (50 to 100 bp) from twelve samples were assembled into 7138 scaffolds ($\geq$4 kb) from which 86601 ORFs were called using FragGeneScan.[2] To these ORFs, 4161, 4543, and 4783 ORFs from strains HCF1, HCN1, and HCS1 were added, respectively. The final list of 100,088 sequences was used to provide candidates for the microarray design. We used ORF length, primary annotation from the m5nr database[3] and metagenome depth of coverage to filter the list of candidate sequences. Metagenome reads from each sample were mapped to the list of predicted ORFs using Bowtie[4]. For each ORF, normalized coverage from each sample was calculated as RPKM measures using custom R scripts. 9939 ORFs whose primary annotation was not deemed to be of biogeochemical interest were filtered. The remaining ORFs were filtered based on the maximum RPKM across 12 samples with the objective of reducing the list of candidate ORFs to fit into the NimbleGen array format. The final list of ORFs represented on the microarray was 44,915. Probes were 60-mers with 7 probes representing each ORF and technical duplicates included in the 630,000-feature region.

References

1. Beller, H. R.; Han, R.; Karaoz, U.; Lim, H.; Brodie, E. L., Genomic and physiological characterization of the chromate-reducing, aquifer-derived Firmicute *Pelosinus* sp. strain HCF1. *Appl. Environ. Microbiol.* **2013,** *79*, (1), 63-73.
2. Rho, M.; Tang, H.; Ye, Y., FragGeneScan: predicting genes in short and error-prone reads. *Nucleic Acids Res* **2010,** *38*, (20), e191.
3. Wilke, A.; Harrison, T.; Wilkening, J.; Field, D.; Glass, E. M.; Kyrpides, N.; Mavrommatis, K.; Meyer, F., The M5nr: a novel non-redundant database containing protein sequences and annotations from multiple sources and associated tools. *BMC Bioinformatics* **2012,** *13*, 141.
4. Langmead, B.; Trapnell, C.; Pop, M.; Salzberg, S. L., Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol.* **2009,** *10*, (3), R25.

Figure S1: Effluent data for individual denitrifying columns.

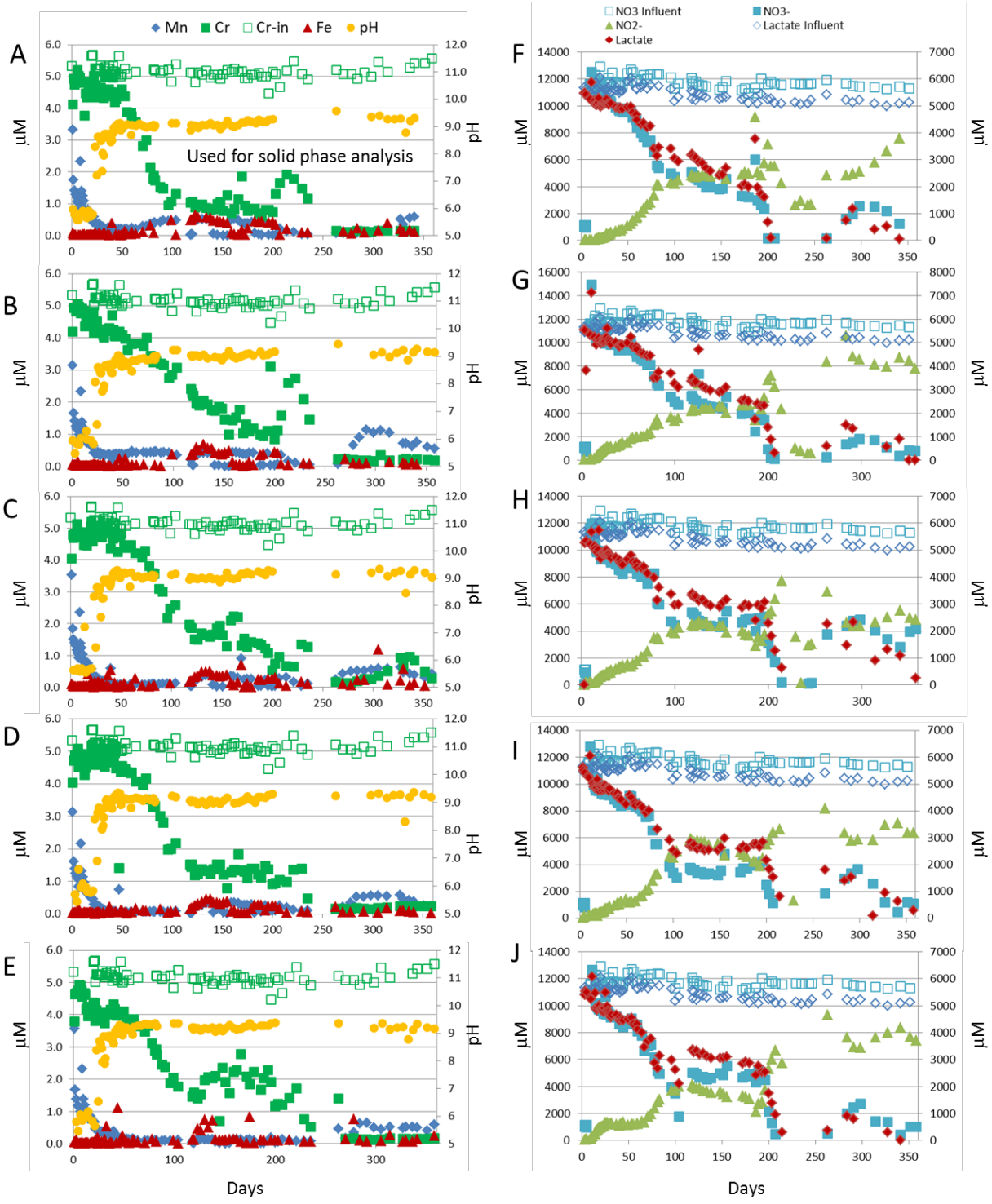


Figure S2: Correlation between chromate reduction and nitrate reduction in all denitrifying columns (mean data plotted) over the first ~100 days of the experiment, when nitrate reduction rate showed the greatest change.

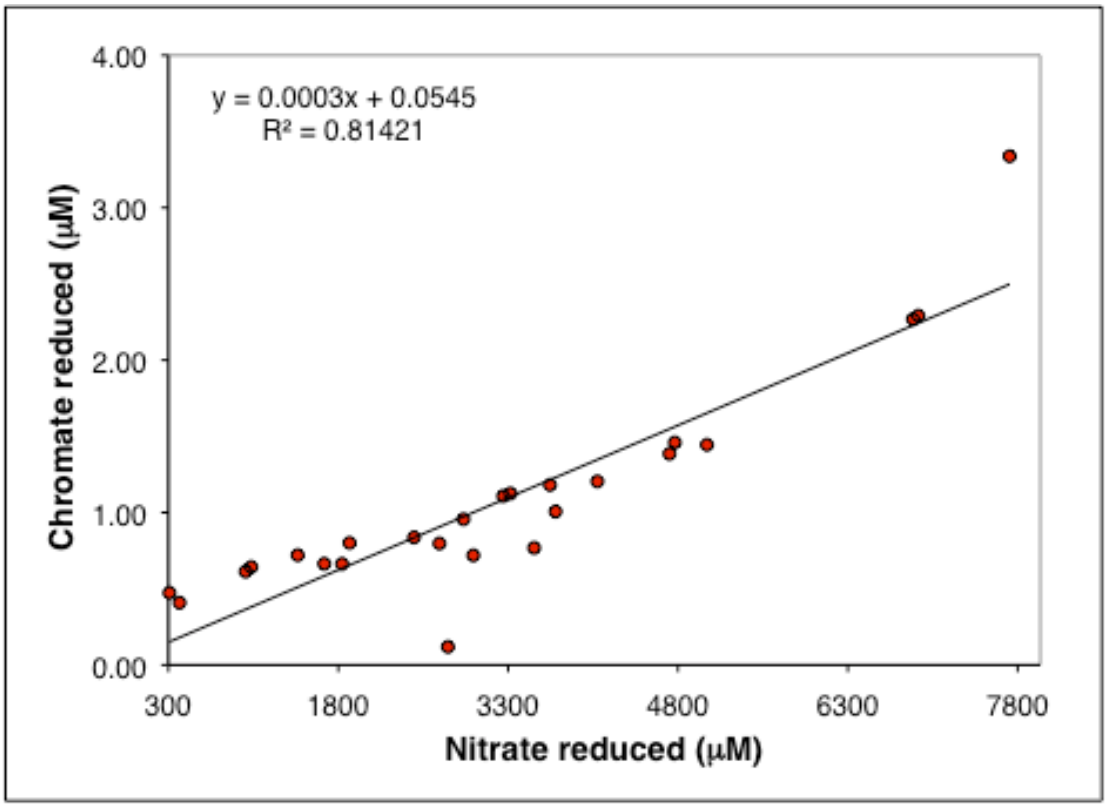


Figure S3: Effluent data for two individual fermentative columns.

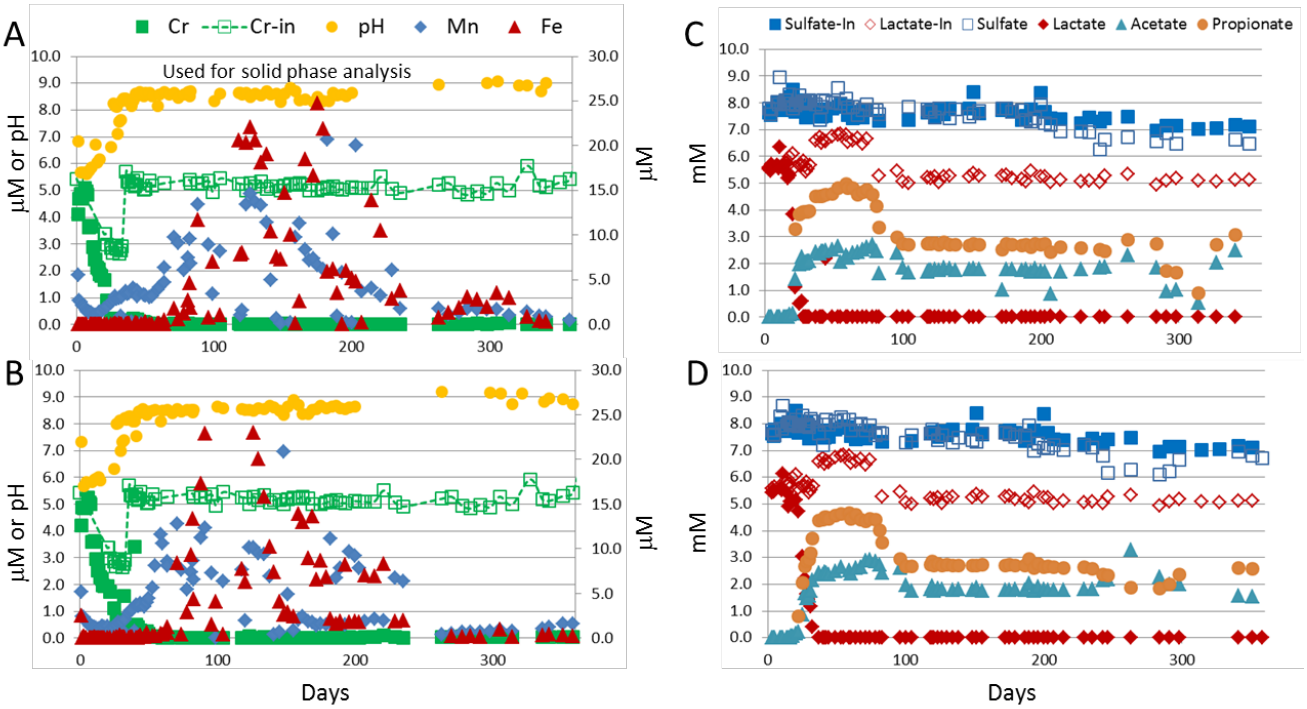


Figure S4: Effluent data for four individual low-activity sulfate-amended columns.

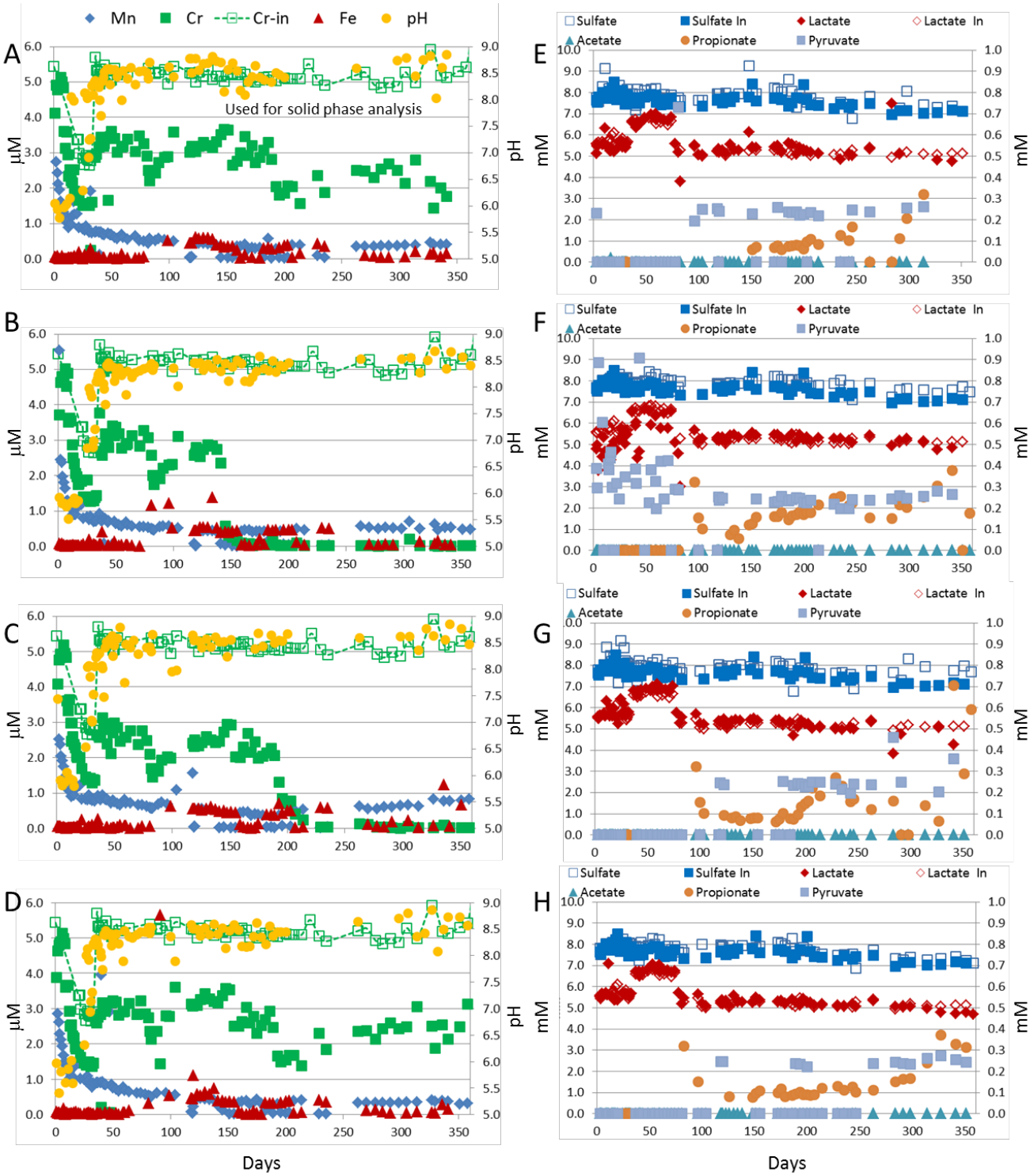


Figure S5: Effluent data for three individual no-added-electron-acceptor columns.

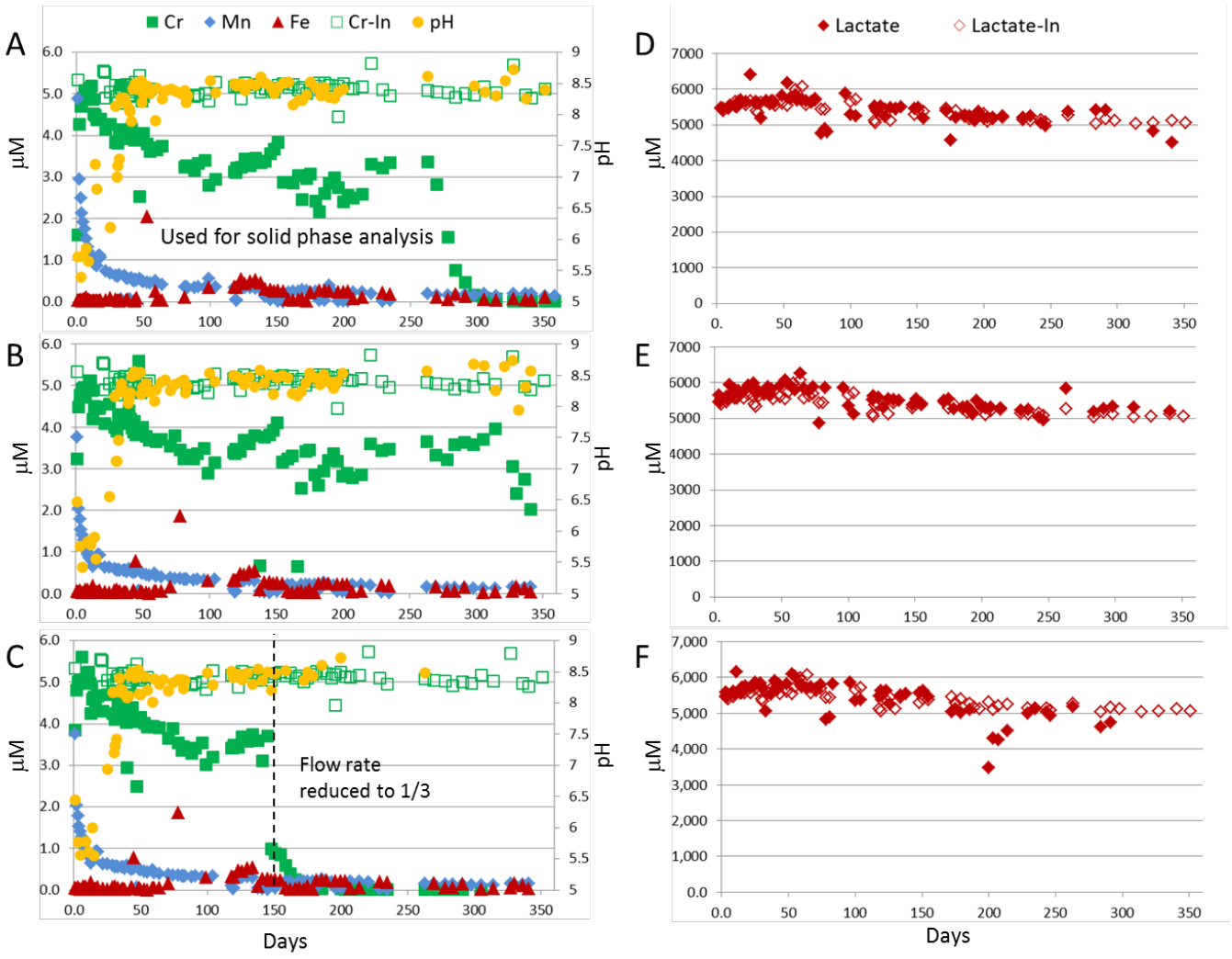


Figure S6: Acid-extractable chromium from the bottom (inlet) sections of four different column types and unincubated column material, analyzed after ~3 and 12 months of incubation.

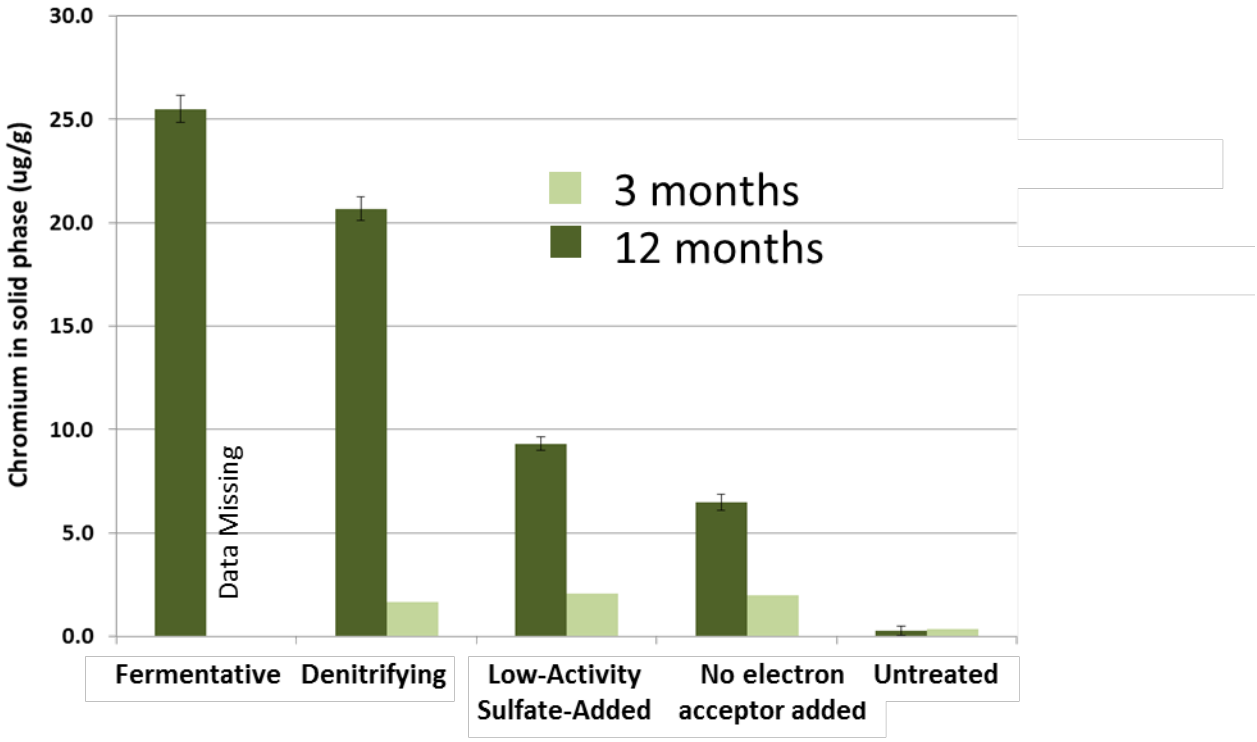


Figure S7: Sulfur K-edge micro-XANES spectra of selected S hot-spots from the Fermentative column showing the presence of reduced sulfur species (e.g. S^0, S^{-1}, S^{-2}) as indicated by the shaded region

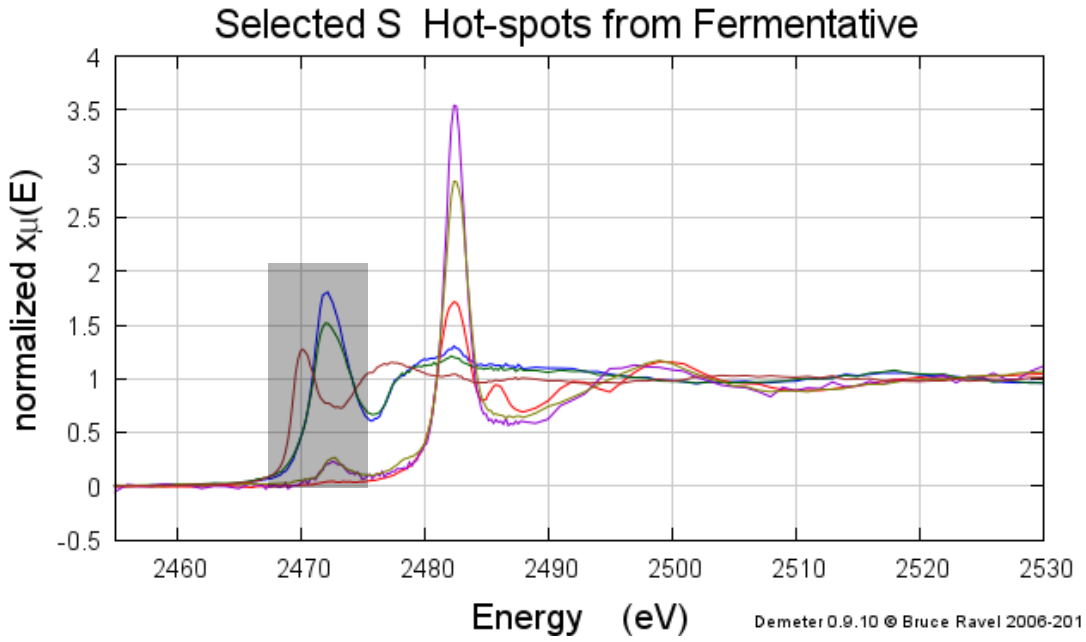


Figure S8: Cr K-edge micro-XANES of multiple Cr hot-spots from a denitrifying column. A few spots show trace amounts of unreduced Cr(VI) as indicated by the brown line marking the pre-edge feature at ~5994.5eV.

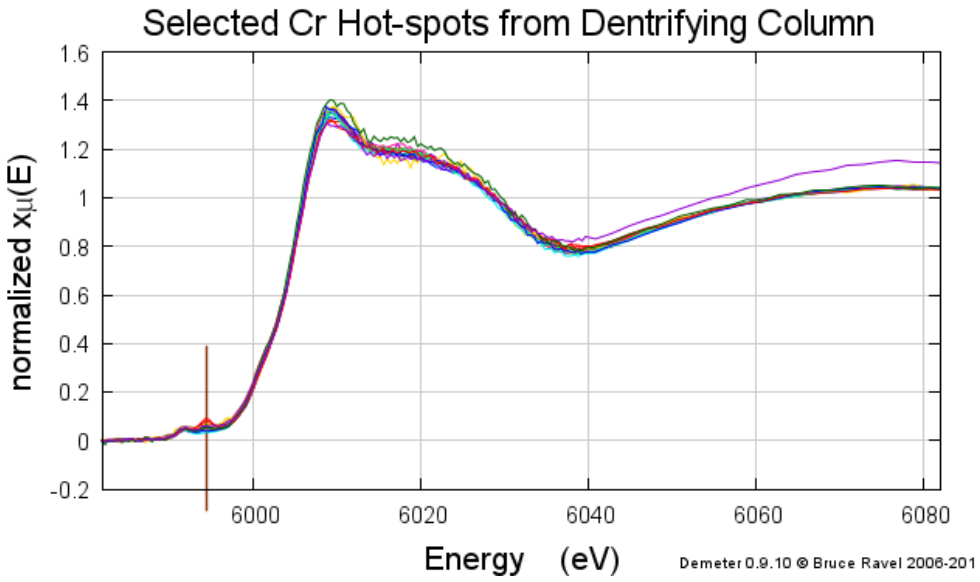
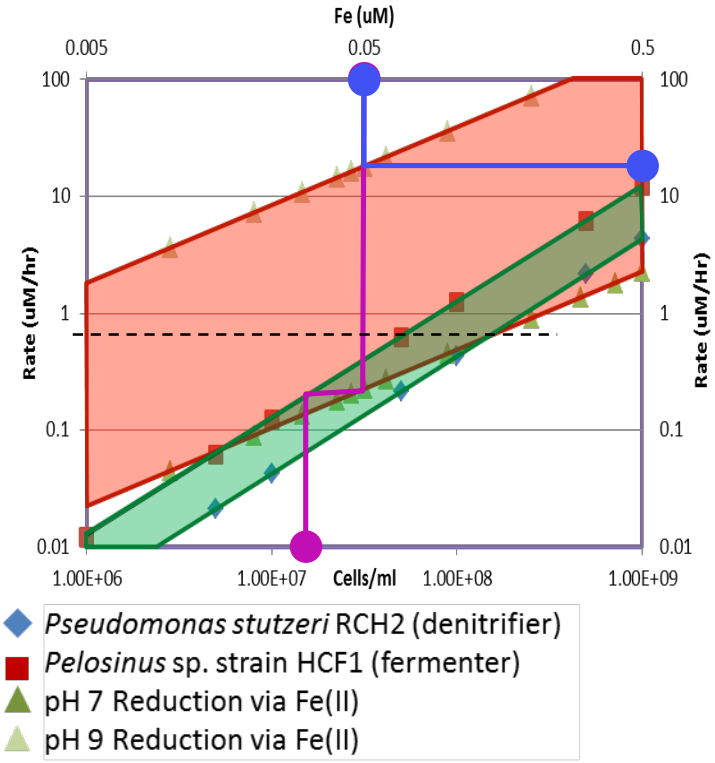


Figure S9: Kinetic comparison of abiotic Cr(VI) reduction by Fe(II) and enzymatic reduction by Hanford 100H aquifer bacteria.



The above figure compares the relative rates of Cr reduction by Fe(II)[1] and two Cr(VI)-reducing organisms isolated from Hanford Site aquifer material (strains HCF1[2] and RCH2[3]). For all calculations, the Cr concentration is assumed to be the same as in the column influent solution, 5 µM. The red field is defined by two lines showing Cr(VI) reduction rate (*y*-axis) as a function of Fe(II) concentration (top *x*-axis) at pH 9 and pH 7, top and bottom lines, respectively, which covers the range of measured pH in the column experiments. The green field is defined by the rate as a function of biomass for strains HCF1 and RCH2, top and bottom lines, respectively. The microbial reduction rate is assumed to be independent of pH for the purposes of this analysis. The blue line is a hypothetical example showing that at a 0.05 µM Fe(II) concentration and pH 9, the rate of Cr(VI) reduction by Fe(II) is expected to be ~10 µM/hr. The purple line illustrates that at the same Fe(II) concentration and pH 7, the Cr(VI) reduction rate is predicted to be similar to a strain HCF1 biomass density of ~$1x10^7$ cells/mL. This plot doesn't account for the biomass needed to reduce Fe(III), which could be comparable to the amount needed to reduce Cr(VI) if one considers solid-phase Fe(III). The dotted line marks ~0.2 µM Cr(VI)/hr rate, which would be sufficient to reduce 5 µM Cr(VI) completely within the column assuming an ~24-hr residence time and a constant rate of reduction (not a valid assumption but used for mathematical simplicity).

1. Pettine, M.; Barra, I.; Campanella, L.; Millero, F. J., Effect of metals on the reduction of chromium(VI) with hydrogen sulfide. *Water Research* **1998,** *32*, 2807-2813.

2. Beller, H. R.; Han, R.; Karaoz, U.; Lim, H.; Brodie, E. L., Genomic and physiological characterization of the chromate-reducing, aquifer-derived Firmicute *Pelosinus* sp. strain HCF1. *Appl. Environ. Microbiol.* **2013,** *79*, (1), 63-73.

3. Han, R.; Geller, J. T.; Yang, L.; Brodie, E. L.; Chakraborty, R.; Larsen, J. T.; Beller, H. R., Physiological and transcriptional studies of Cr(VI) reduction under aerobic and denitrifying conditions by an aquifer-derived pseudomonad. *Environ. Sci. Technol.* **2010,** *44*, (19), 7491-7.